Original article

Pre-transplant factors influencing HLA crossmatch in deceased donor kidney transplantation

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Abstract:

Introduction: *Factor affecting the waiting list for deceased donor kidney transplantation is the retrieval time of the donor's organs until a transplant kidney has been given to the patient. Hence, the delivery of the spleen and/or lymph nodes to the laboratory must be kept in the transport medium at the appropriate temperature. Besides, the HLA crossmatch testing time is another factor affecting kidney transplantation.* ***Objective:*** *To study retrospective information about factors affecting the HLA crossmatch test for kidney transplantation from deceased donors.* ***Materials and Methods:*** *The numbers of kidneys that could not be transplanted and the duration of the HLA crossmatch test using complement-dependent cytotoxicity (CDC) and CDC-Antihuman globulin (CDC-AHG) for kidney transplantation from deceased donors to the patient candidates on waiting list of the Organ Donation Centre and the National Blood Centre, Thai Red Cross Society, to receive a kidney transplant were studied.* ***Results:*** *The total number of deceased donors was 1,233 cases with an upward trend every year. Fourteen samples from the deceased donors (1.13%) could not be used for HLA crossmatch test, owing to cell low viability (n = 13, 1.05%) and cell insufficiency for testing (n = 1, 0.08%). Moreover, the duration of testing time was approximately 5 to 6 hours, starting from obtaining the spleen and/or lymph nodes of the deceased donors to the time that the laboratory reported the results to the Organ Donation Centre.* ***Conclusion:*** *The important factors affecting the HLA crossmatch time before deceased donor kidney transplantation including not only specimen transportation but also the quality of laboratory management would be beneficial to support the increasing number of deceased kidney donors.*

Keywords : ● *HLA crossmatch* ● *Deceased donors* ● *Kidney transplantation*

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Introduction

In Thailand, organ sharing for kidney transplantation from deceased donors has been organized by the Organ Donation Centre, The Thai Red Cross Society with the cooperation of the Division of Special Laboratory (Special Lab), National Blood Centre (NBC), The Thai Red Cross Society since 1997. Human leukocyte antigen (HLA) crossmatch is performed for registered patients. The deceased donor kidneys will be allocated to the patients with the highest scores which derived from number of HLA mismatched, panel reactive antibody (PRA), waiting time in the list and the age of the patients[1].

The donor blood samples were tested for HBsAg, anti-HBs, anti-HBc, anti-HCV, HIV-Ag/Ab, syphilis, anti-CMV IgG and IgM by chemiluminescense microparticle immunoassay (CMIA) and HIV-RNA, HBV-DNA and HCV-RNA by nucleic acid testing (NAT) by Special Lab, NBC. If all of results were acceptable, we performed and recorded ABO, Rh(D), and HLA-A, -B, -DR typing. The results were entered into the computer software for matching. The selection criterias were based on ABO identical, least HLA antigen mismatched and no antibody against donor HLA antigens[2,3]. The patients on waiting list are requested to collect and send their monthly serum to NBC. After obtaining a rank-order of candidates to be offered each kidney generated from the computer software. The selected patient monthly sera were then set for HLA crossmatch against donor T and B lymphocytes. The donated kidneys will be allocated to those who got highest scores with negative HLA crossmatch only[2,3].

The main factor influencing the outcome of kidney transplantation is the cold ischemic time (CIT). Even though, after harvesting and keeping the kidneys in the organ preserved medium at 4°C, the cell damage still occur regarding inflammatory and immune responses. Then it is necessary to control the CIT to be as short as possible in order to prevent delayed graft function (DGF). It was indicated that less than 30 hours CIT will not effect the long-term graft survival[4-6]. In addition, it was observed that CIT that over 30 hours may be 40% at risk for graft failure as compared to CIT of 6 hours[7].

Patel and Terasaki reported in 1969[8] that hyperacute rejection correlated well with incompatible HLA crossmatch by complement-dependent cytotoxicity (CDC) technique. At present, CDC technique is considered as gold standard for HLA crossmatch. However, cytotoxicity test greatly depends on the viability of the test cells. The low viability cells could yield false positive result. The low viability cells may be due to inappropriate temperature for storage and transportation of blood samples and the effect of some drugs taken by the donor. Therefore, it is recommended that the lymphocytes from the donor spleen and lymph nodes should be used for HLA crossmatch. The yield and viability of lymphocytes are better than lymphocytes obtained from peripheral blood. It is recommended that for HLA crossmatch, the viability of test cells should be over 85%[9]. The United Network for Organ Sharing (UNOS) guidelines recommended RPMI 1640 or minimum essential medium (MEM) for transport medium to preserve the lymphocytes for HLA crossmatch. Besides, one should be aware of bacterial contamination by not using outdated medium and should add antibiotic to the medium if being reopened and recapped several times. In addition, the transport temperature in container with ice should be controlled at 4°C[10].

In the routine practice, after receiving the specimens, the laboratory staff will manage to prepare the T and B lymphocytes and select serum samples of 8-10 highest-score patients in the waiting list for HLA crossmatch[11]. The standard turn around time (TAT) for HLA crossmatch was 9-10 hours[9]. The TAT greatly depends on the quality of test lymphocytes and the skill of the personnel performing the test. The aim of this study was to analyze retrospective information concerning factors affecting TAT of HLA crossmatch test for kidney transplantation from deceased donors.

Materials and Methods

Population included in this study

Retrospective study of HLA crossmatch for kidney transplant from deceased donors for patients in the waiting list of the Organ Donation Centre and the Special laboratory, NBC from January 1, 2014 to December 31, 2019.

Methods

Data consisted of general information of deceased-kidney donors such as gender, age and number of transplanted kidneys, TAT of HLA crossmatch and number of kidneys which could not be transplanted. The methods for HLA crossmatch were CDC lymphocytotoxicity test (CDC) and CDC-Antihuman globulin (CDC-AHG). The CDC method: the selected patients sera were tested against deceased donor T and B lymphocytes at 4°C, 22°C and 37°C. After adding complement to the test, if the patient possessed antibody corresponding to the donor HLA antigen will result in cell lysis which can be shown by adding Eosin dye (5%). The percentage of dead cells can be examined under inverted phase contrast microscope. The grading of the reactions was as follows: < 10%, 10-20%, 20-40%, 40-60%, 60-100% which were assigned as 1, 2, 4, 6 and 8, respectively. The scores of 2 to 4 were considered as negative, whereas the scores of 4 to 8 were positive[12]. The positive and negative control serum must be tested along with the test samples each time. So the test lymphocyte samples should be more than 85% viability in order to be valid for the test. The CDC-AHG method is quite similar to CDC method only adding antihuman globulin serum (AHG) to washed cells after each incubation temperature. Then the complement was added to each well and the rest of the procedure was the same as CDC[9]

Analysis of the data

The comparison of the data during six-year period of study was performed on deceased kidney donors regarding the percentage of their gender and age. In addition to the number of the patients receiving kidney transplantation, information on specimen receiving and TAT of HLA crossmatch. One-way ANOVA (Dunnett T3) was applied using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA). The statistically significant difference will be considered when *p-value* < 0.05.

Result

Retrospective study of the data of Organ Donation Centre, The Thai Red Cross Society during January 1, 2014 to December 31, 2019, there were a total of 1,465 deceased donors[13] (Table 1). However, only 1,233 cases were subjected to perform the HLA crossmatch by the Special Lab, NBC. Among 1,219 cases (98.86%) which could be tested for HLA crossmatch, they were 974 males (79.90%) and 245 females (20.10%). The age of the donors were 4 months to 80 years. The frequency of male-donor age group was as follows: 41-50 years

Table 1 Number of deceased donors and kidney transplant patients reported by the Organ Donation Centre, Thai Red Cross Society[13]

Year	Deceased donors	Kidney transplant patients	Kidney transplantation: Deceased Donor
2014	188	330	1.7
2015	204	364	1.8
2016	220	414	1.9
2017	293	543	1.8
2018	260	473	1.8
2019	300	552	1.8
Total	1,465	2,676	1.8

Table 2 Age and sex of deceased donors from January 1, 2014 to December 31, 2019 (n = 1,219)

Age (years)	Number (%)	
	Male	Female
0-10	12 (1.20)	8 (3.30)
11-20	150 (15.40)	23 (9.40)
21-30	133 (13.70)	21 (8.60)
31-40	219 (22.50)	44 (18.00)
41-50	256 (26.30)	64 (26.10)
51-60	166 (17.00)	66 (26.90)
61-70	38 (3.90)	17 (6.90)
71-80	0 (0.00)	2 (0.80)
Total	974 (79.90)	245 (20.10)

256 cases (26.30%), 31-40 years 219 cases (22.50%) and 51-60 years 166 cases (17.00%), respectively. While the frequency for females donors was 51-60 years 66 cases (26.90%). The next orders were 41-50 years, 64 cases (26.10%) and 31-40 years, 44 cases (18.00%) (Table 2).

The data indicated that in six-year period from 2014 to 2019, the trend of deceased kidney donors was increased, i.e.188 cases in 2014 and increased to 300 in 2019. However, the ratio between the number of transplanted patients to the number of deceased kidney donors were between 1.7 to 1.9 (Table 1). Fourteen of 1,233 cases, the specimens could not be used for HLA crossmatch (1.13%) which mainly due to low viability of the cells.

Among 13 cases (1.05%) there were 3, 4, 3, 2 and 1 cases of low viability cells which happened in the year 2014, 2015, 2016, 2017 and 2018, respectively. In addition of 1 case (0.08%) with low yield of lymphocytes in the year 2014. This was due to insufficient lymphocytes for the unexpected additional HLA crossmatch request for the new set of patients, since only one of the selected patients from the first set of HLA crossmatch could be transplanted, while the rest of them had health problem.

The TAT in the six-year period was 3:40 hours. to 9:52 hours. The majority was 5-6 hours (Figure 1). The increased trend of the deceased donors was observed, i.e. during the year 2014 to 2016, the deceased donors were less than 200 cases, while they were 248, 168 and 266 in 2017,2018 and 2019, respectively.

It was found that the average shortest TAT of HLA crossmatch was 370.89 min. in 2018 while, the average longest TAT was 393.66 min. in 2017. The average time of the TAT for the year 2015 was shorter than 2017 statistically significant difference ($p < 0.05$). In addition, TAT of HLA crossmatch in 2018 was shorter than 2017 statistically significant difference ($p < 0.05$) (Table 3).

Discussion

Factors affecting DGF post kidney transplantation from deceased donors: for patient factors, i.e. body mass index, immunological factors and yet it depends on quality of the donated kidneys which correlated with age and serum creatinine of the kidney donors. For the storage and transportation of harvested kidneys. It had been reported that every hour incretion of CIT will increase DGF of 4%[14-16]. In order to reduce our TAT of HLA crossmatch, from 2000 to 2007 HLA typing for deceased donors at our center using serological technique and since July 2007 to present, it was changed to PCR technique. This technique enables us to decrease the time for testing and the result interpretation, including requires less blood samples[17].

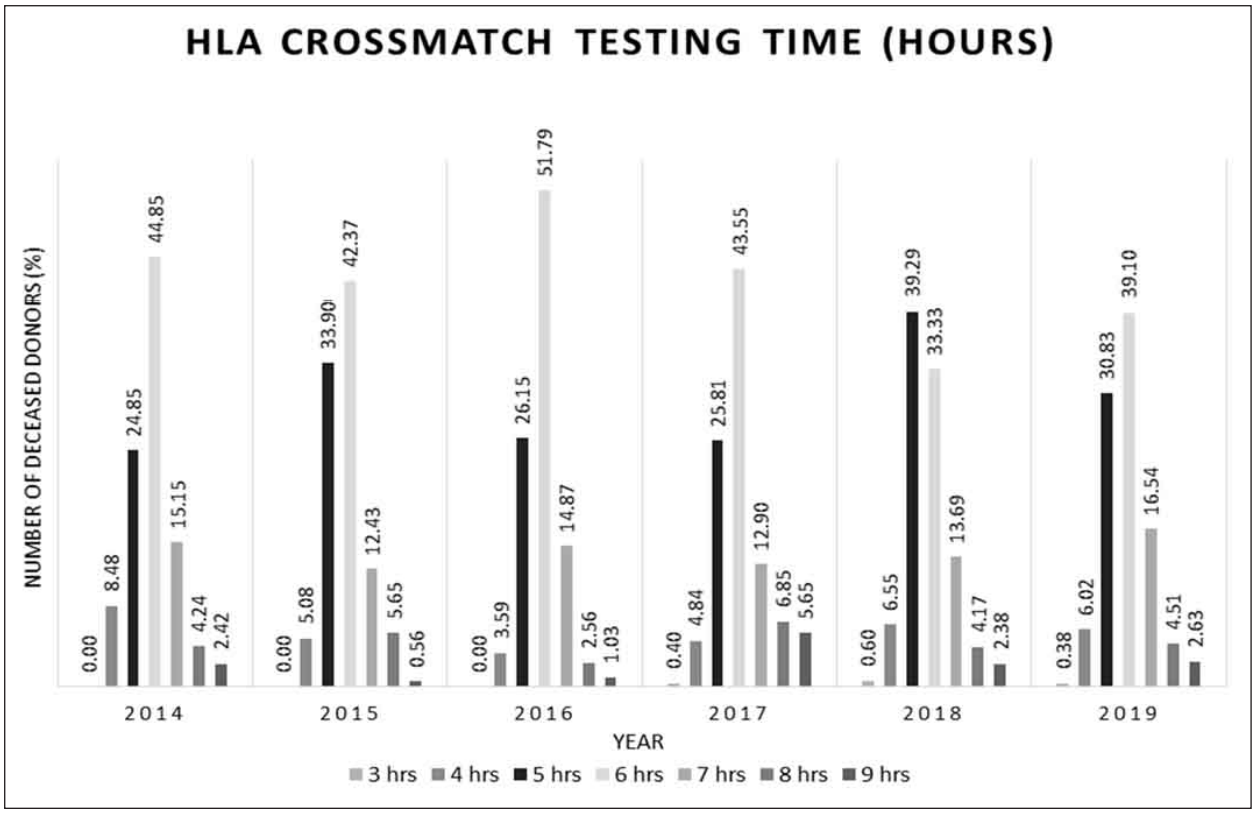

Figure 1 Percentage of cases according to various TAT from January 2014 to December 2019

Table 3 The spread out and evaluate variability of TAT

Year	Number of donors	HLA crossmatch testing time (minutes)			
		Minimum	Maximum	Mean	SD
2014	165	240	560	378.68	60.43
2015	177	240	548	375.01[a]	55.37
2016	195	260	560	381.03	49.50
2017	248	221	585	393.66[a,b]	69.53
2018	168	221	580	370.89[b]	63.30
2019	266	235	592	381.43	61.44

[a,b] = $p < 0.05$

The number of male deceased donors were four times higher than female deceased donors and majority of age group was between 21 to 60 years (males 79.5% and females 86.5%). This finding was in agreement with previous report regarding deceased kidney donors and the death statistics on the road traffic in Thailand[10].

Even though, the number of cell low viability in this study was small (1.05%), the cause should be identified and eliminated. In this study, it was found that the cases of low viability were mainly due to the wrongly use of normal saline as media during transportation of spleen and lymph nodes. It had been reported that the use of normal saline instead of RPMI 1640 or MEM may result in cell low viability. In addition, the container should be maintained at 4°C in order to avoid bacterial contamination[9,11]. The request for the new set of patients for HLA crossmatch may occur unexpectedly, especially when the selected patients were cancelled due to health problem. The laboratory should be well prepared to spare sufficient lymphocytes for unforeseen situation.

The main factor to reduce CIT problem is to reduce the laboratory testing time which is the HLA typing for the deceased kidney donor. At present, the donor admitted hospital will send deceased kidney donor blood samples to the laboratory. Then the laboratory is able to select the serum from the patients on waiting list who had highest score to prepare and ready for HLA crossmatch. This protocol should result in shortening the TAT of HLA crossmatch. In this study, the longest TAT was 9:52 hours. This was due to there were 3 deceased donors at the same time. The laboratory had developed queuing system according to the kidney harvesting times from the donors. There was a study indicated that to shorten the CIT by reducing the HLA crossmatch incubation time to be 3-4 hours will result in low sensitivity of the test[18]. However, in this study, the TAT of HLA crossmatch did not exceed 9-10 hours (ASHI standard)[9]. The average of TAT in 2017 was statistically significant higher than 2015 and 2018. This may be due to the increase in number of deceased donors in 2017 which increased to 248 cases. So the management of the personnel and laboratory work process efficiently will be crucial for the laboratory to maintain the TAT according to the standard.

Conclusion

This study reported the factors influencing HLA crossmatch for kidney transplantation from deceased donors. The important factors were the quality of the specimens which should be kept in medium under the control temperature for storage and transportation. In addition, the laboratory should be able to manage to cope with the increasing in number of the deceased donors each year and the control of TAT for HLA crossmatch. All of these factors will reduce the occurrence of DGF post-kidney transplantation from deceased donors.

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